Biochimica et Biophysica Acta 1817 (2012) S115–S126

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

S15 Mitochondrial Physiology

15L1

ROS as signaling molecules in mitochondrial and cellular (dys)function

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Cell regulation by signaling ROS ("sROS") is often incorrectly studied through extracellular oxidant addition. Recently we used the membrane-permeable antioxidant Trolox to examine the role of sROS in mitochondrial morphology, oxidative phosphorylation (OXPHOS) and cytosolic calcium (Ca^{2+}) handling in healthy human skin fibroblasts [1]. Trolox treatment reduced the levels of CM-H_2DCF oxidizing ROS, lowered cellular lipid peroxidation and induced a less oxidized mitochondrial thiol redox state. This was paralleled by increased glutathione- and mitofusin-dependent mitochondrial filamentation, increased expression of fully-assembled mitochondrial complex I, elevated activity of citrate synthase and OXPHOS enzymes, and a higher cellular O_2 consumption. In contrast, Trolox did not alter hydroethidium oxidation, cytosolic thiol redox state, mitochondrial NAD(P)H levels or mitochondrial membrane potential. Whole genome expression profiling revealed that Trolox did not trigger significant changes in gene expression, suggesting that Trolox acts downstream of this process. Cytosolic Ca^{2+} transients, induced by the hormone bradykinin, were of higher amplitude and decayed faster in Trolox-treated cells. These effects were dose-dependently antagonized by hydrogen peroxide. Our findings suggest that Trolox-sensitive sROS are upstream regulators of mitochondrial mitofusin levels, morphology and function [3] in healthy human skin fibroblasts. This information not only facilitates interpretation of antioxidant effects in cell models (of oxidative-stress), but also contributes to a better understanding of ROS-related human pathologies including mitochondrial dysfunction [2,4,5].

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doi:10.1016/j.bbabio.2012.06.308

15L2

Insights into mitochondrial structure and function from correlated four-dimensional light and electron microscopy

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Earlier studies of mitochondrial ultrastructure by three-dimensional electron microscope tomography established the crista junction paradigm of inner mitochondrial membrane conformation in which tubular inner membrane cristae that vary in length among different "species" of mitochondria connect lamellar cristae with the inner boundary membrane via crista junctions of uniform diameter. Although this complex membrane conformation may be a thermodynamically stable one, the uniform crista junction sizes and the lengths of tubular cristae appears to be sensitive to the presence of proteins such as OPA1 and ATP synthase. The crista junction structural model does not hold for certain types of mitochondria or during some cellular processes such as apoptosis. Through correlated light and electron microscopic studies, both two- and three-dimensional, of HeLa cells during asynchronous apoptosis, we have characterized some structural changes that occur during release of cytochrome *c*. Most notable is the conversion of the normal inner membrane into many separate vesicular matrix compartments. This structural change does not, however, appear to be required for complete release of cytochrome *c*, but does provide clues to the possible topological mechanism of inner membrane fission and fusion and to the physical parameters that control mitochondrial inner membrane conformation.

doi:10.1016/j.bbabio.2012.06.309

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Conformational change of mitochondrial complex I as a key event in ischaemia/reperfusion damage

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Myocardial ischaemia/reperfusion is associated with mitochondrial dysfunction followed by the cardiomyocyte death. If ischaemia is followed by rapid reperfusion it may cause cellular dysfunction and tissue injury. Mitochondria are recognized as key organelle involved in post-ischemic reperfusion damage.

Mitochondrial complex I can exist in two interconvertible states: active (A) and de-active or dormant (D). We have studied the A/D equilibrium in several tissues after in vivo induced ischaemia and investigated the sensitivity of both forms of the enzyme to reactive oxygen species (ROS).

We performed analysis of the A/D ratio of mitochondrial complex I obtained from various tissues after the onset of cardiac arrest. We found that in highly metabolising tissues such as heart and brain the $t_{1/2}$ of complex I de-activation is 12 and 2.5 min, respectively. Reperfusion resulted in the return of complex I A/D equilibrium to its initial level, indicating that reintroduction of oxygen causes reactivation of the D-form of the enzyme. The generation of excessive quantities of ROS and subsequent tissue damage is an important mechanism of reperfusion injury. We found that only the D-form of complex I was susceptible to inhibition by ROS whereas the A-form of the enzyme was insensitive to either hydrogen peroxide or superoxide. Turnover-dependent activation of the preparation D-form of the enzyme resulted in formation of the A-form which was much less sensitive to ROS. The nature of complex I modification by ROS is likely to involve oxidation of cysteine thiols. The D-form of complex I modified in this way does not catalyse the physiological NADH:ubiquinone reaction, making this enzyme a mitochondrial target for oxidative stress and an early target in ischaemia/reperfusion damage. Therefore, transition of complex I into the dormant form in the absence of oxygen is critical to the process of cardiac injury during ischaemia/reperfusion and may have important pathophysiological consequences.

doi:10.1016/j.bbabio.2012.06.310

15L4

ROS production by respiratory complex II

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A common observation is that the rate of H_2O_2 production is remarkably higher under succinate oxidation than under NADH-linked (glutamate/pyruvate) oxidation in isolated rat brain, heart and skeletal muscle mitochondria. As the H_2O_2 production driven by succinate oxidation is strongly inhibited by the CI inhibitor rotenone, it is generally believed that succinate oxidation produces ROS via a "reverse electron flow" from CII to CI. However, oxidation of submillimolar (physiological) concentrations of succinate can produce H_2O_2 even in the presence of high concentrations of NAD-dependent substrates or NADH, which makes the presumed CII–CI reverse electron flow more thermodynamically unfavorable.

Moreover, although rotenone potently block ROS production driven by succinate oxidation, approximately 10–15% of the initial ROS remains, which cannot be catalyzed by CI $FMNH_2$. In addition, this ROS fraction may be significantly higher (1.3–15 times) than the ROS produced by the CI forward reaction. The simpler explanation for the generation of the rotenone-insensitive ROS driven by succinate oxidation is that they are produced by the CII redox components. However, it is surprising to find that CII contribution to ROS production has been mostly disregarded and hence has not been experimentally assessed. In the present study, experiments were designed to undoubtedly establish that CII indeed significantly contributes to the generation of ROS.

doi:10.1016/j.bbabio.2012.06.311

15L5

Functional characterization and regulation of UCP4 expression by adipokinetic hormone in larva and pupa fat body mitochondria from the beetle *Zophobas atratus*

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Uncoupling protein 4 (UCP4) is the member of the large family of mitochondrial anion transporters that uncouple oxidative phosphorylation [1]. The present study demonstrates for the first time, the molecular identification of a partial *Zophobas atratus* UCP4 coding sequence and the functional characterisation of ZaUCP4 in the mitochondria of larval and pupal fat bodies of the beetle. ZaUCP4 shows a high similarity to predicted insect UCP4 isoforms and known mammalian UCP4s, both at the nucleotide and amino acid sequence levels. Bioenergetic studies unequivocally demonstrate UCP4 activity in mitochondria isolated from larvae and pupae fat body. In resting, non-phosphorylating state 4 respiration ZaUCP activity was stimulated by palmitic acid and inhibited by the purine nucleotide GTP. In phosphorylating mitochondria, ZaUCP4 activity decreased the yield of oxidative phosphorylation. ZaUCP4 was immunodetected by using of antibodies raised against human UCP4 as a single 36 kDa band. Because it is known that hormones influence an expression of UCPs, we tested adipokinetic hormone (AKH), analog of mammal glucagon, which mobilizes lipids and carbohydrates from fat body stores [2]. Besides this energy-mobilizing function, AKHs inhibit fat body lipid and protein synthesis. After AKH injection, we observed the decrease in ZaUCP4 expression at the mRNA and protein levels in both developmental stages of the beetle.

Real time analysis, immunological detection and bioenergetic characteristic indicate consistently for the higher expression of UCP4 in the *Z. atratus* larval fat body compared with the pupal fat body, furthermore ZaUCP4 expression is under hormonal control. The different expression patterns and activity of ZaUCP4 during the larval–pupal transformation indicate an important physiological role for UCP4 in insect fat body development and function during insect metamorphosis. Furthermore, the regulation of UCP4 expression by AKH indicates that it may play a role in maintaining metabolic homeostasis in the insect.

This work was supported by the grant of the Polish Ministry of Science (N N 303291634).

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doi:10.1016/j.bbabio.2012.06.312